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1973-Plat
Imaging of Lipid Bilayer Mixtures and Actual Cell Membrane Fragments by Nanosims
Monica M. Lozano, Steven G. Boxer.
Stanford University, Stanford, CA, USA.
Lipids, proteins, and cholesterol are the major components of biological membranes; however, little is known about their lateral organization and how it changes during cell-cell interactions, signaling, development and division on the nanometer length scale that is relevant for function. These studies develop a new method for determining the spatial composition and organization of model membranes using secondary ion mass spectrometry (SIMS) imaging in an effort to explain the rich phase behavior of complex biological membranes. Furthermore, these experiments set the stage for measuring the native distribution of cholesterol and proteins within membrane fragments taken from cells. Advanced fluorescence and atomic force microscopies (FM and AFM, respectively) used thus far to interrogate model cell membranes have limited molecular specificity (can only detect fluorescently labeled molecules in FM), spatial resolution (governed by optics in FM or tips in AFM) and both lack compositional information. SIMS imaging, in particular using the NanoSIMS by Cameca, offers imaging capability with high sensitivity (sub-ppm), specificity (based on isotopic labeling), and spatial resolution (~50nm). Using this unique technique, the composition and organization of cholesterol, lipids and proteins within the plane of the lipid bilayer of model and actual cell membranes with nanometer resolution is investigated. Cholesterol in plasma membranes is responsible for modulating acyl chain order, membrane elasticity and lateral organization and its level is tightly regulated. In order to investigate the spatial distribution of cholesterol in actual cell membranes, supported cell membrane fragments are imaged using the NanoSIMS. These measurements are the first example of the direct analysis of the organization of a cell membrane at the nanometer length scale that is relevant for function setting the stage for quantitative analysis of systems of increasing complexity and direct biological relevance.

1974-Plat
Near-Critical Fluctuations and Cytoskeleton-Assisted Phase Separation Lead to Subdiffusion in Cell Membranes
Eugene P. Petrov, Jens Ehrig, Petra Schwille.
Technische Universitaet Dresden, Dresden, Germany.
We address the relationship between membrane microheterogeneity and anomalous subdiffusion in cell membranes by carrying out large-scale lattice-based Monte Carlo simulations of two-component lipid membranes. We study the diffusion of lipid molecules in free membranes and find that near-critical fluctuations in the membrane lead to transient subdiffusion of lipid molecules spanning several orders of magnitude in time [1, 2]. We observe that the membrane-cytoskeleton interaction strongly affects phase separation, enhances subdiffusion, and eventually leads to hop diffusion of lipids [2]. Thus, we present a minimum realistic model for membrane rafts showing the features of both microscopic phase separation and subdiffusion.
[1] J. Ehrig, E. P. Petrov, and P. Schwille, arXiv:1009.4860.
[2] J. Ehrig, E. P. Petrov, and P. Schwille, arXiv:1010.1207.

Platform AS: Voltage-gated K Channels - Gating II

1975-Plat
The Phenylalanine in the Gating Charge Transfer Center is Critical for Modulating the Rate of Voltage Sensor Movement in Shaker Potassium Channels
Stephan A. Pless, Christopher A. Ahern.
University of British Columbia, Vancouver, BC, Canada.
Voltage-gated potassium channels contribute to cellular excitability by selectively gating the transmembrane passage of potassium ions. The homologous residue to Phe290 in Shaker potassium channels is invariably an aromatic side chain in voltage-gated ion channels. Interestingly, a recent study has suggested that aromaticity at this position is not required for WT-like function. Furthermore, tryptophan substitutions, together with lysine substitutions of the charge-carrying arginines in S4 can dramatically affect voltage-sensor movement. However, it remains unclear how individual mutations at position 290 alone affect voltage-sensor movements. Given the close physical proximity of Phe290 and the voltage sensor, we hypothesized that mutations at Phe290 should affect the rate of voltage sensor movement. To test this hypothesis we replaced Phe290 by the smaller, hydrophobic side chains, Ala and Leu, and monitored voltage sensor movement with gating current recordings in HEK293 cells and voltage-clamp fluorometry experiments in Xenopus oocytes. Surprisingly, our results show that hydrophobic, non-cyclic side chain substitutions at position 290 do not change the voltage dependence of the voltage sensor movement (i.e. QV and FV). However, the ON component of gating currents and fluorescence readout from reporter groups on the top of S4 are substantially slowed. As voltage-gated sodium channels display dramatically faster gating kinetics than potassium channels we next sought to investigate if aromaticity is required at the homologous positions in one or more of the four domains of the skeletal sodium channel. We employed in vivo non-sense suppression to incorporate fluorinated Phe derivatives in all four domains. However, fluorination had only minor effects on the voltage-dependence of activation and steady-state inactivation, suggesting that, similar to Shaker potassium channels, aromaticity is not absolutely required in these positions in sodium channels.

1976-Plat
Inactivation and Voltage-Dependent Rectification Mechanisms in the KcsA Potassium Channel
Celine Boiteux, Simon Bernèche.
Biozentrum, University of Basel, Basel, Switzerland.
Potassium channels regulate ion permeation by varying their conductance notably through a mechanism known as C-type inactivation, which implies that shortly after activation, their selectivity filter stops conducting ions at rates that depend on various stimuli. This inactivation process plays a critical role in controlling the length and frequency of cardiac action potentials, as well as the firing patterns in neurons.
It's been shown that the prokaryotic KcsA channel undergoes C-type inactivation like its eukaryotic counterparts (Gao et al., PNAS 102:17630 (2005)), suggesting KcsA as a prototypic model for structural studies of inactivation gating. The detailed microscopic process underlying C-type inactivation remains unexplained despite the accumulation of experimental evidences showing the key role played by the selectivity filter and some neighboring residues. In particular, the interactions between Asp80, Glu71 and Trp67, as well as the water molecules trapped in the P-loop of the channel, seem to be strongly linked to the stability of the filter and its ability to adopt a stable inactivated state (Cordero-Morales et al., Nat. Struct. & Mol. Biol. 14:1062 (2007)). Models of inactivation gating that were proposed on the basis of x-ray crystallography studies involve transitions between conducting states, containing two ions, and non conducting ones, containing a single ion (Zhou et al., Nature 414:23 (2001); Cuello et al., Nature 466:272 (2010)).
Using molecular dynamic simulations and free energy calculations, we investigated the possible transitions between different ion occupancy states involving the conducting and putatively inactivated conformations of KcsA. A comparative study of key mutants showing different inactivation phenotypes allows us to propose a structural model describing the inactivation mechanism of KcsA, as well as the voltage dependent rectification observed in WT and some mutants.

1977-Plat
The Second Highly-Conserved Threonine Residue of the Potassium Channel Signature Sequence Mediates Activation-Coupled to C-Type Inactivation
Luis G. Cuello, Dominique G. Gagnon, D. Marien Cortes.
Texas Tech University Health Science Center, Lubbock, TX, USA.
In the amino-acid sequence of a K+ channel pore domain are encoded most of the structural elements necessary to 1) catalyze the permeation of K+ ions and 2) execute activation and C-type inactivation gating. In KcsA, a C-type inactivation process similar to that found in eukaryotic channels has been identified [1] and crystallographic evidences indicate that the collapse of the channel selectivity filter (SF) is responsible for it [2]. It was also established that an allosteric communication between the channel activation gate and the SF underlies activation coupled to C-type inactivation in KcsA and other K+ channels [3]. In KcsA, F103 seems to mediate this allosteric communication by interacting with T75 of the same subunit, the second highly conserved Threonine in the SF of K+ channels (T**T**XGYGD). In order to gain functional and structural insights regarding this allosteric communication, we have mutated KcsA-T75 to a Glycine or an Alanine and

evaluated the functional consequences of severing this molecular interaction. The resulting phenotype for these perturbations was a non-inactivating one, albeit having altered conduction properties (smaller single-channel conductance). Additionally, a similar phenotype was observed for the *Shaker* K+ channel when the equivalent position T442 was mutated to either Gly or Ala, suggesting that a common mechanism underlies activation coupled to C-type inactivation in K+ channels. Finally, the X-ray solution structure for these KcsA mutants trapped in the open state, will be presented and discussed in the context of activation coupled to C-type inactivation in K+ channels.

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1)Cordero-Morales, J.F., et al. Nat Struct Mol Biol, 2006. **13**(4): p. 311-8.
2)Cuello, L.G., et al. Nature, 2010. **466**(7303): p. 203-8.
3)Cuello, L.G., et al.. Nature, 2010. **466**(7303): p. 272-5.

1978-Plat

On the Structural Basis of Modal Gating Behavior in K^+ Channels

Sudha Chakrapani[1], Julio F. Cordero-Morales[2], Vishwanath Jogini[3], Albert C. Pan[4], D Marien Cortes[5], Benoît Roux[6], Eduardo Perozo[6].
[1]Case Western Reserve University, Cleveland, OH, USA, [2]University of California San Francisco, San Francisco, CA, USA, [3]D. E Shaw Research, Hyderabad, India, [4]D. E Shaw Research, NY, NY, USA, [5]Texas Tech University, Lubbock, TX, USA, [6]University of Chicago, Chicago, IL, USA.

Modal-gating shifts represent an effective regulatory mechanism by which ion channels control the extent and time course of ionic fluxes. Under steady-state conditions, the K^+ channel KcsA displays three distinct gating modes, high-P_o, low-P_o and a high-frequency flicker mode, each with about an order of magnitude difference in their mean open times. In KcsA, the hydrogen bond network between Glu71, Asp80 and Trp67 that surrounds the selectivity filter has been shown to regulate C-type inactivation, with the Glu71-Asp80 pair having the strongest influence on selectivity filter stability. Here, we show that in the absence of C-type inactivation, mutations at the pore-helix position Glu71 unmask a series of kinetically distinct modes of gating in a side-chain-specific way which mirror those seen in wild-type channels. Results from high-resolution crystal structures along with molecular dynamic simulations suggest that specific interactions in the side-chain network surrounding the selectivity filter, in concert with ion occupancy, alter the relative stability of pre-existing conformational states of the pore. These findings highlight the key role of the selectivity filter in regulating modal gating behavior in K^+ channels.

1979-Plat

Mode Shift of the Voltage Sensors in Shaker K^+ Channels is Caused by Energetic Coupling to the Pore Domain

Georges A. Haddad, Rikard Blunck.
Université de Montréal, Montreal, QC, Canada.

The voltage sensors (S1-S4) of Kv channels are known to undergo a conformational change when triggered by membrane depolarisation that subsequently leads to opening of the central pore (S5-S6) and ion conduction. The electromechanical coupling between these domains is mediated by two well conserved regions: the C-terminus of the S6 segment and the S4-S5 linker. Upon change in the membrane potential, the structural rearrangements induced by the charged S4 segment can be measured as gating currents. During prolonged depolarization, voltage-gated ion channels present a behaviour called the ''mode-shift''. This shift causes the QV to change towards negative potentials and essentially affects the energy required by the system to bring all sensors back to their resting state. To understand the structural cause of this process, we investigated the coupling between the pore and voltage sensor and its influence on the development of the mode shift. In our approach we used the cut-open voltage clamp fluorometry method to simultaneously measure gating or ionic currents and conformational changes of the S4 segment. We identified mutations that fully uncouple voltage sensors from the pore domain thus eliminating the mutual influence of these two domains on one another. The results show that, when eliminating the coupling between these domains, the voltage sensors require less energy to move. At the same time the mode shift occurring during prolonged depolarization is abolished. By instead preventing open state stabilization, voltage sensors are kept in the opposite mode. We also found that the mode shift is accompanied by a conformational change in the S4 segment. We propose that the pore influences the voltage sensor by the presence of a ''mechanical load'' and allosterically induces a conformational change in the S4.

1980-Plat

A shaker Potassium Channel with a Miniature Engineered Voltage Sensor

Yajamana Ramu, Yanping Xu, Zhe Lu.
University of Pennsylvania, Philadelphia, PA, USA.

Voltage-gated ion channels sense transmembrane voltage changes via a paddle-shaped motif that includes the C-terminal part of the third transmembrane segment (S3b) and the N-terminal part of the fourth segment (S4) that harbors voltage-sensing arginines. Here, we find that residue triplets in S3b and ^{NT}S4 can be deleted individually, or even in some combinations, without compromising the channels' basic voltage-gating capability. Thus, a high degree of complementarity between these S3b and ^{NT}S4 regions is not required for basic voltage gating per se. Remarkably, the voltage-gated Shaker K+ channel remains voltage gated after a 43 residue paddle sequence is replaced by a glycine triplet. Therefore, the paddle motif comprises a minimal core that suffices to confer voltage gating in the physiological voltage range, and a larger, modulatory part. Our study also shows that the hydrophobic residues between the voltage-sensing arginines help set the sensor's characteristic chemical equilibrium between activated and deactivated states.

1981-Plat

A Converging Consensus of the Structure of a Voltage-Sensing Domain in its Resting State

Ernesto Vargas, Francisco Bezanilla, Benoit Roux.
University of Chicago, Chicago, IL, USA.

Understanding the voltage-sensing mechanism of Kv+ channels requires gaining knowledge of the active and resting conformations of the voltage-sensing domain (VSD). However, the lack of structural information remains an obstacle in revealing the activation pathway between conformations. Although crystallographic studies have provided atomic resolution structures of Kv+ channels in their putative open/inactive state, no crystal structure of a Kv+ channel in the resting state is currently available. In the absence of complete atomic resolution structures, experimental and computational studies have sought to complement the structural information of the channel in both conformations. Despite efforts to generate a consistent model of the resting state no consensus has been reached. In the present work, we have probed the accuracy of Khalili-Araghi's model of a VSD in the resting state by subjecting it to experimentally-determined structural constraints. These include metal bridges between transmembrane helices S1/S4, S2/S4, S2/S3, and an interaction between S2/S4 that spatially position the transmembrane helices with respect to each other. Of particular importance, we simulated the actual mutations and interactions that were involved in the referenced experiments. The purpose of these simulations has been to address the compatibility of Khalili-Araghi's VSD model with the available experimental data. The simulations demonstrate that a rough consensus exists on the overall conformation of the resting state of the VSD when all available experimental information is utilized.

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1982-Plat

Voltage-Gated Potassium Ion Channels are Frustrated

Philip W. Fowler, Mark S.P. Sansom.
University of Oxford, Oxford, United Kingdom.

It has been suggested for some time that prolines allow the S6 helices of voltage-gated potassium ion channels to move between straight and kinked conformations thereby gating the channel. The kinked conformation was subsequently observed in the x-ray crystallographic structures of Kv1.2 and the paddle chimera, both of which are open. Although closed structures of potassium ion channels with straight inner helices have been observed, none of these have the characteristic PVPV motif. When the voltage sensor moves in response to changes in transmembrane potential, it pulls on the S4-S5 linker, moving this also, which in turn presses on the S6 helix and closes the channel. What remains unclear in this mechanical model is how the channel then opens. We shall show using free energy calculations that the S6 helices of the paddle chimera prefer to be kinked and that conformations corresponding to a closed channel are not favoured. Our results suggest that when the channel is open the S6 helices adopt similar conformations to those observed in the x-ray crystallographic structures. These calculations include the pore region of the paddle chimera, a lipid bilayer and explicit water and therefore include the steric and cooperative effects introduced by moving all four S6 helices simultaneously. We conclude that the closed state of the paddle chimera is frustrated; the S6